Renal Nerves Promote Sodium Excretion in Angiotensin-Induced Hypertension

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Abstract—To determine whether the sympathetic nervous system contributes to the hypertension induced by pathophysiological increments in plasma angiotensin II (Ang II) concentration, we determined the neurally induced changes in renal excretory function during chronic intravenous infusion of Ang II. Studies were carried out in five conscious chronically instrumented dogs subjected to unilateral renal denervation and surgical division of the urinary bladder into hemi-bladders to allow separate 24-hour urine collection from the denervated and innervated kidneys. After control measurements, Ang II was infused for 5 days at a rate of 4.8 pmol/kg per minute (5 ng/kg per minute), this was followed by a 5-day recovery period. Twenty-four-hour control values for mean arterial pressure (MAP) and for the ratio of denervated to innervated kidneys (DEN/INN) for urinary sodium, potassium, and creatinine excretion were 93±5 mm Hg, 117±0.09, 110±0.10, and 100±0.02, respectively. As expected, Ang II infusion caused sodium retention for several days before sodium balance was achieved at an elevated MAP (day 5=124±4 mm Hg). Moreover, by day 2 of Ang II-induced hypertension, there were significant reductions in the DEN/INN for sodium and potassium, which persisted for the 5 days of Ang II infusion. On day 5, the DEN/INN values for sodium and potassium were 0.71±0.10 and 0.91±0.12, respectively. In contrast, the DEN/INN for creatinine was unchanged from control levels during Ang II infusion, and measurements of renal hemodynamics indicated comparable reductions in glomerular filtration rate (~13%) and renal plasma flow (~25%) during Ang II infusion. This indicates that the renal nerves promoted sodium and potassium excretion during Ang II-induced hypertension by inhibiting tubular reabsorption of these electrolytes. Thus, this study provides no support for the hypothesis that increased renal sympathetic nerve activity impairs sodium excretion and contributes to Ang II-induced hypertension (Hypertension. 1998;31[part 2]:429-434.)

Key Words: angiotensin II • hypertension • kidney • sympathetic nervous system • renal nerves • sodium

The renal-angiotensin system plays an important role in both short and long-term regulation of arterial pressure. Because numerous studies have shown that Ang II stimulates sympathetic activity by both central and peripheral actions, there has been considerable interest in the possibility that the sympathetic nervous system contributes to the cardiovascular effects of circulating Ang II. However, the physiological and pathophysiological significance of the interactions between the renal-angiotensin and sympathetic nervous systems is not fully understood. It has been particularly difficult to discern whether the sympathetic nervous system plays a role in mediating the long-term hypertensive effects of circulating Ang II.

There is considerable theoretical and experimental evidence that the kidneys play a vital role in long-term regulation of arterial pressure. Long-term control of arterial pressure is achieved by the renal body fluid feedback mechanism whereby the kidneys slowly regulate arterial pressure by altering body fluid volumes by the effect of pressure on sodium excretion, referred to as pressure-natriuresis. Theoretically, the renal nerves could link the sympathetic nervous system to long-term volume and arterial pressure control by altering pressure-natriuresis. Indeed, experimental studies have shown that chronic renal adrenergic stimulation shifts the pressure-natriuresis relationship to a higher level of arterial pressure. Therefore, if the sympathetic nervous system contributes to the long-term hypertensive effects of Ang II, one would expect hypertension induced by high circulating levels of Ang II to be associated with increased renal sympathetic nerve activity and neurally induced sodium retention. Unfortunately, because of difficulty in monitoring long-term changes in renal sympathetic nerve activity and assessing the functional effects of the renal nerves under chronic conditions, there is little direct support for this hypothesis.

The split-bladder preparation combined with unilateral renal denervation is a powerful technique for exposing a functional role of the renal nerves because both kidneys are exposed to the same perfusion pressures and hormonal influences. Consequently, any differences in sodium excretion between the kidneys can be attributed to either the direct or indirect effects of the renal nerves on renal excretory function. In the present study, we used the powerful split-bladder technique combined with unilateral renal denervation to test the hypothesis that the renal nerves promote sodium retention in hypertension induced by pathophysiological circulating levels of Ang II.

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Selected Abbreviations and Acronyms

Ang II = angiotensin II
DEN/INN = denervated/innervated ratio
GFR = glomerular filtration rate
MAP = mean arterial pressure
PRA = plasma renin activity

Methods

Animal Preparation

Five female dogs weighing 18 to 20 kg were used in this study, and all procedures were in accordance with National Institutes of Health guidelines and approved by the Institutional Animal Care and Use Committee. Before surgery, the dogs were administered atropine (0.05 mg/kg SC), sedated with acepromazine (0.15 mg/kg SC), and then anesthetized with either pentobarbital sodium (25 mg/kg IV) or sodium thiopentone (1.5% to 2.5%). Catheters made of Tygon microcure tubing were implanted in the lower abdominal aorta and inferior vena cava via the femoral arteries and veins, respectively, and exteriorized through the scapulae. Subsequently, the urinary bladder was surgically divided, and each half was sutured to form hemibladders with Silastic catheters implanted to allow continuous 24-hour urine collection from each kidney. The catheters were exteriorized in the flank region and connected to sterile plastic bags. Finally, the left kidney was denervated through a flank approach. All visible nerves along the renal artery and vein were removed, the adventitia was stripped, and the vessels were cannulated with arterial and venous catheters was maintained by flushing with isotonc (buprenorphine hydrochloride, 0.015 mg/kg IM BID). Patency of the catheters was confirmed by flushing catheters, calibrating blood pressure transducers, feeding, and cleaning cages.

Experimental Protocol

During the 10-day training and equilibration period, the dogs were trained to lie quietly in their cages for measurement of renal function and collection of blood samples. After a 3-day control period, the dogs were continuously infused with Ang II ([Asp'Val']angiotensin II, Ciba-Geigy Corp) for 5 days by adding the peptide to the 24-hour saline infusion. Ang II was infused at a rate of 4.8 pmol/kg per minute (5 ng/kg per minute) which increases plasma levels to ~5 times control. The chronic infusion of Ang II was followed by a 5-day recovery period. Renal function was measured on the last day of the control, experimental, and recovery periods. On these days, 5-mL arterial blood samples were taken for determination of hematocrit, PRA, and the plasma concentrations of sodium, potassium, and protein. Throughout the study, urine samples were collected daily for determination of 24-hour urinary excretion rates of sodium, potassium, and creatinine.

GFR and renal plasma flow were estimated from the clearances of [125I]iothalamate (Glofil, Cypress Pharmaceuticals Corp) and [131I]iodohippurate (Hippuran, Symbion International Corp), respectively, as previously described. During each experiment, the results of three consecutive 20-minute clearance periods were averaged to determine the GFR and renal plasma flow. At the end of the clearance periods, each hemibladder was flushed twice with a total of 20 mL of sterile distilled water, and the wash was added to the urine collected. A 1.5-mL arterial blood sample was taken at the midpoint of each clearance period for determination of the plasma concentrations of [125I]iothalamate and [131I]iodohippurate.

Analytical Methods

PRA was measured by radioimmunoassay. Plasma and urine concentrations of sodium and potassium were determined by flame photometry (Il 943, Instrumentation Laboratory), plasma protein concentration by refractometry (American Optical), and hematocrit by a microtiter method (Autocrit II, Clay Adams). Urinary creatinine concentration was determined with a creatinine analyzer (model 2, Beckman).

Statistical Analysis

Results are expressed as mean±SEM. Experimental and recovery data were compared with control using analysis of variance with Dunnett's t test for multiple comparisons. Statistical significance was considered to be P<0.05. The relative excretion rates of sodium, potassium, and creatinine from denervated and innervated kidneys are expressed by the ratio DEN/INN.

Results

Fig 1 shows that chronic infusion of Ang II produced hypertension in the absence of a significant reduction in heart rate. MAP increased ~20 mm Hg during the 1st day of Ang II infusion and on day 5 was elevated 31±3 mm Hg above control (control=93±5 mm Hg). During this time, PRA (control=0.42±0.17 nmol Ang I·L⁻¹·h⁻¹) decreased to undetectable levels, and plasma potassium concentration (control=3.4±0.1 mmol/L) fell 0.2±0.1 mmol/L. There were no significant changes in hematocrit (control=37±3%) or in the plasma concentrations of either sodium (control=144±1 mmol/L) or protein (control=6.7±0.3 mg/dL) on day 5 of Ang II infusion. Recovery values for all of the above were similar to control levels.
Figure 1. Effects of chronic infusion of Ang II on mean arterial pressure and heart rate. Values are mean±SEM; n=5. *P<.05.

Fig 2 illustrates the changes in sodium excretion during chronic Ang II infusion. As expected, Ang II caused sodium retention for several days before daily sodium balance was achieved; however, for the 5-day period of Ang II infusion, there was no significant net retention of sodium. As illustrated by the DEN/INN for sodium excretion (control=1.17±0.09, ≈15 to 20% more sodium was excreted from denervated than innervated kidneys during the control period. Moreover, and most importantly, this ratio reversed during Ang II infusion. By day 2 of Ang II infusion, there was a significantly greater rate of sodium excreted from innervated than denervated kidneys, and this response persisted throughout the remainder of the Ang II infusion period; during days 2 to 5 of Ang II infusion, sodium excretion was ≈35% higher in innervated than denervated kidneys. On day 5, the DEN/INN for sodium excretion was 0.71±0.10. This indicates that the renal nerves promoted sodium excretion during Ang II-induced hypertension. The DEN/INN for sodium excretion returned to control levels during the recovery period.

Although there was no significant change in potassium balance during Ang II infusion, the DEN/INN for potassium excretion decreased in parallel with the DEN/INN for sodium excretion (Fig 3). During the control period, ≈10% more potassium was excreted from denervated than innervated kidneys (control DEN/INN=1.10±0.10), but on days 2 to 5 of Ang II infusion, the relative excretion rates changed as innervated kidneys excreted 10 to 15% more potassium than denervated kidneys. On day 5, the DEN/INN for potassium excretion was 0.91±0.12. Thus, the renal nerves promoted the excretion of potassium, as well as sodium, during Ang II-induced hypertension. During the recovery period, the DEN/INN for potassium excretion returned to control levels.

As illustrated in Fig 4, during Ang II infusion there were no significant changes in total creatinine excreted each day. Further, in marked contrast to the fall in the DEN/INN for sodium and potassium excretion during Ang II infusion, there were no significant changes in the DEN/INN for creatinine excretion at this time (control DEN/INN=1.00±0.02). This indicates that there were comparable changes in GFR in denervated and innervated kidneys during Ang II infusion.

Finally, the Table summarizes the renal hemodynamic responses to chronic Ang II infusion in denervated and innervated kidneys. During the control period, the values for GFR, renal plasma flow, and filtration fraction were similar in denervated and innervated kidneys. Moreover, the renal hemodynamic responses to chronic infusion of Ang II were comparable in both kidneys. On day 5 of Ang II infusion, GFR and renal plasma flow were reduced ≈13 and 25%, respectively, whereas filtration fraction was elevated ≈20%. Although apparently still suppressed during the recovery period, neither GFR nor renal plasma flow was significantly different from control at this time.

Discussion

The major novel finding of the present study was that the renal nerves promoted sodium excretion, not sodium retention, during hypertension produced by pathophysiological plasma levels of Ang II. Thus, this study provides no support for the hypothesis that increased renal sympathetic nerve activity impairs sodium excretion and contributes to Ang II-hypertension. Rather, the present results, along with those from earlier studies discussed below, indicate that suppression of renal
sympathetic nerve activity is a chronic compensatory response, which may actually attenuate the antinatriuretic and hypertensive effects of Ang II.

The relative increase in sodium excretion in innervated versus denervated kidneys during Ang II-hypertension is consistent with the results from several studies which indicate that renal sympathetic nerve activity is suppressed during chronic infusion of Ang II at rates which produce hypertension. In a study in chronically instrumented dogs, Carroll et al. reported that renal norepinephrine overflow (an index of renal sympathetic nerve activity) was depressed after 6 days of Ang II-hypertension. A subsequent study by Vari et al. showed that renal norepinephrine content was reduced in rats infused with high levels of Ang II for 14 days. Most recently, Cox and Bishop directly recorded renal sympathetic nerve activity in conscious rabbits subjected to Ang II-induced hypertension. They found that renal sympathetic nerve activity was depressed after 10 days of Ang II infusion. Given the central role of the kidneys in long-term regulation of body fluid volumes and arterial pressure and the possibility that sustained alterations in renal sympathetic nerve activity may chronically alter pressure natriuresis, the above studies are particularly relevant to the hypothesis that the sympathetic nervous system contributes to Ang II-hypertension because of the emphasis on changes in renal sympathetic nerve activity. Because regional sympathetic activity is differentially regulated, it cannot be assumed from global indices of sympathetic function (such as plasma levels of norepinephrine) or from sympathetic activation to other organs that a comparable pattern of sympathetic activation occurs in the kidneys. Therefore, taken in the context of these earlier studies, the present results can be interpreted to indicate that chronic suppression of renal sympathetic nerve activity promotes sodium excretion during chronic Ang II infusion and, therefore, neurally induced sodium retention is not a mechanism that contributes to the hypertension.

The present findings also demonstrate that the predominant long-term effects of the renal nerves on sodium excretion are mediated via actions on tubular function. Measurements of renal hemodynamics under resting conditions indicated that GFR decreased during Ang II infusion and that the fall in GFR (and renal plasma flow) was comparable in denervated and innervated kidneys. In addition to these measurements, the relative 24-hour excretion rates of creatinine in denervated and innervated kidneys were unchanged during Ang II infusion, indicating that reductions in GFR were similar in both kidneys throughout the entire day. Thus, the present findings suggest that the renal nerves chronically impair sodium reabsorption during Ang II-induced hypertension and, therefore, are consistent with the results from acute studies which indicate that small changes in renal sympathetic nerve activity, including those mediated by baroreflexes, influence sodium reabsorption in the absence of changes in GFR and renal plasma flow. Further, if the proximal tubule is the predominant site of neurally induced alterations in sodium reabsorption under chronic as well as acute conditions, then an increased rate of sodium delivery to the distal nephron could account for the greater excretion of potassium in innervated than denervated kidneys during Ang II infusion in the present study.
Other studies have been interpreted to support the hypothesis that chronic infusion of Ang II produces hypertension, at least in part, by increasing sympathetic activity. First, two studies in dogs have reported that Ang II is more effective in causing hypertension when chronically infused into the vertebral artery than when administered intravenously. Because infusion of Ang II into the vertebral artery produces high concentrations of Ang II at the area postrema, a circumventricular organ in the medulla oblongata that apparently mediates many of the cardiovascular effects of Ang II, these studies have been taken as evidence that the central actions of Ang II to increase sympathetic activity contribute to Ang II-induced hypertension. However, a recent study by Hildebrandt et al. in dogs failed to confirm the contention that greater arterial pressure was recorded 24 hours/d and pathophysiological (not pharmacological) levels of Ang II were achieved in the circulation. Second, surgical ablation of the area postrema has been reported to totally abolish the hypertension induced by chronic intravenous infusion of Ang II. However, ablation of the area postrema also abolished deoxyxycorticosterone-salt hypertension and markedly attenuated the development of hypertension in the spontaneously hypertensive rat. Because the activity of the renin-angiotensin system is either normal or suppressed in these latter two models of hypertension, the antihypertensive effects of area postrema lesions appear to be nonspecific. Furthermore, lesions of the area postrema produce anorexia and weight loss for several weeks after surgery, as well as other disturbances in salt and water metabolism. A recent study by Collister et al. indicated that alterations in food intake per se after lesions of the area postrema influence the cardiovascular responses to the renin-angiotensin system. Thus, the mechanism by which destruction of the area postrema interferes with chronic Ang II-induced hypertension is not clear and appears to be due to more than simply loss of central Ang II receptors. Finally, a number of investigators have assessed sympathetic activity during chronic Ang II infusion by acute administration of ganglionic blockers at various time points throughout the progression of Ang II-hypertension. Because there is a progressive increase in the hypertensive response to acute ganglionic blockade during the evolution of the hypertension, it has been concluded that the sympathetic nervous system plays an increasingly important role in mediating the hypertension. However, it is important to recognize that there are probably a number of factors other than the level of sympathetic activity (eg, initial level of arterial pressure and volume status) that influence the hypertensive response to ganglionic blockade. Moreover, this study design does not directly assess the influence of the sympathetic nervous system on the slowly acting renal blood-fluid feedback mechanism, which is the dominant long-term controller of arterial pressure. Thus, these acute blocking studies may not be relevant to long-term blood pressure control. Indeed, Hall and Granger reported that chronic ganglionic blockade reduced arterial pressure considerably more in normal dogs than in dogs with Ang II-induced hypertension, suggesting that chronic Ang II-induced hypertension may have induced sympathetic activity. In summary, there is rather meager evidence that the sympathetic nervous system contributes to Ang II-induced hypertension.

In conclusion, the results of the present study are novel because they clearly demonstrate a sustained influence of the renal nerves on sodium excretion during Ang II-induced hypertension and, moreover, suggest that the sympathetic nervous system may play a role in long-term regulation of sodium excretion and arterial pressure via changes in renin sympathetic nerve activity. Taken in the context of our earlier observations that renal norepinephrine overflow is chronically suppressed in Ang II-induced hypertension, the greater excretion rate of sodium from innervated than denervated kidneys during chronic infusion of Ang II indicates that suppression of renal sympathetic nerve activity and, in turn, neurally induced sodium excretion is a compensatory response to the hypertension. This hypothesis is consistent with our recent observations that there is also a relatively greater rate of sodium excretion in innervated than denervated kidneys during hypertension induced by chronic infusion of high rates of norepinephrine. An important goal in future studies will be to identify the afferent mechanisms that lead to chronic renal sympathoinhibition during hypertension. It is acknowledged that the present study does not exclude the possibility that Ang II has direct stimulatory effects on the sympathetic nervous system, which persist chronically but are normally masked by feedback mechanisms that suppress sympathetic outflow. The present study, however, clearly shows that the renal sympathetic nerves do not promote sodium retention and contribute to Ang II-hypertension when all feedback mechanisms are functional.

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References

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